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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Garth J.S. Cooper	Group: 180
Serial No.:	236,985	Art Unit: 189B
Filed :	August 26, 1988	Examiner: Lester L. Lee
For :	TREATMENT OF DIABETES MELLITUS	

SUBMISSION OF PRIORITY DOCUMENT PURSUANT TO 35 U.S.C. §119

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Transmitted herewith for filing in the above-identified application is a certified copy of United Kingdom patent application No. 8720115, for which Applicants claimed foreign priority benefits in their Declaration filed with the PTO on October 26, 1988, in accordance with 35 U.S.C. §119.

Respectfully submitted,

LYON & LYON

Date: 5-1-91 By:

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The Patent Office

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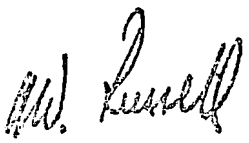
Witness my hand this 16th day of JANUARY 1991



PATENTS ACT 1977

26 AUG 1987

PATENTS FORM No. 1/77 (Revised 1982)

(Rules 16, 19)

The Comptroller
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1987
20115

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REQUEST FOR GRANT OF A PATENT

8720115

THE GRANT OF A PATENT IS REQUESTED BY THE UNDERSIGNED ON THE BASIS OF THE PRESENT APPLICATION

I Applicant's or Agent's Reference *(Please insert if available)* PP/2555

II Title of Invention

TREATMENT OF DIABETES MELLITUS

III Applicant or Applicants *(See note 2)*

Name (First or only applicant) G.J.S.Cooper

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Country State

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IV Inventor (see note 3)

(b) A statement on Patents Form No 7/77 will be furnished

V Name of Agent (if any) *(See note 4)* Stevens, Hewlett & Perkins | ADP CODE NO

VI Address for Service (See note 5) 5 Quality Court
Chancery Lane
London WC2A 1HZ

VII Declaration of Priority (See note 6)

Country	Filing date	File number

VIII The Application claims an earlier date under Section 8(3), 12(6), 15(4), or 37(4) (See note 7)

Earlier application or patent number ..... and filing date .....

IX Check List *(To be filled in by applicant or agent)*

A The application contains the following number of sheet(s)		B The application as filed is accompanied by:	
1 Request	1 Sheet(s)	1 Priority document	
2 Description	8 Sheet(s)	2 Translation of priority document	
3 Claim(s)	2 Sheet(s)	3 Request for Search	
4 Drawing(s)	Sheet(s)	4 Statement of Inventorship and Right to Grant	
5 Abstract	Sheet(s)		

X It is suggested that Figure No ..... of the drawings (if any) should accompany the abstract when published.

XI Signature *(See note 8)* STEVENS, HEWLETT & PERKINS

NOTES:

1. This form, when completed, should be brought or sent to the Patent Office together with the prescribed fee and two copies of the description of the invention, and of any drawings.

2. Enter the name and address of each applicant. Names of individuals should be indicated in full and the surname or family name should be underlined. The names of all partners in a firm must be given in full. Bodies corporate should be designated by their corporate name and the country of incorporation and, where appropriate, the state of incorporation within that country should be entered where provided. Full corporate details, eg "a corporation organised and existing under the laws of the State of Delaware, United States of America," trading styles, eg "trading as xyz company", nationality, and former names, eg "formerly [known as] ABC Ltd." are not required and should not be given. Also enter applicant(s) ADP Code No. (if known).

3. Where the applicant or applicants is/are the sole inventor or the joint inventors, the declaration (a) to that effect at IV should be completed, and the alternative statement (b) deleted. If, however, this is not the case the declaration (a) should be struck out and a statement will then be required to be filed upon Patent Form No 7/77.

4. If the applicant has appointed an agent to act on his behalf, the agent's name and the address of his place of business should be indicated in the spaces available at V and VI. Also insert agent's ADP Code No. (if known) in the box provided.

5. An address for service in the United Kingdom to which all documents may be sent must be stated at VI. It is recommended that a telephone number be provided if an agent is not appointed.

6. The declaraton of priority at VII should state the date of the previous filing and the country in which it was made and indicate the file number, if available.

7. When an application is made by virtue of section 8(3), 12(6), 15(4) the appropriate section should be identified at VIII and the number of the earlier application or any patent granted thereon identified.

8 Attention is directed to rules 90 and 106 of the Patent Rules 1982.

9. Attention of applicants is drawn to the desirability of avoiding publication of inventions relating to any article, material or device intended or adapted for use in war (Official Secrets Acts, 1911 and 1920). In addition after an application for a patent has been filed at the Patent Office the comptroller will consider whether publication or communication of the invention should be prohibited or restricted under section 22 of the Act and will inform the applicant if such prohibition is necessary.

10. Applicants resident in the United Kingdom are also reminded that, under the provisions of section 23 applications may not be filed abroad without written permission or unless an application has been filed not less than six weeks previously in the United Kingdom for a patent for the same invention and no direction prohibiting publication or communication has been given or any such direction has been received.

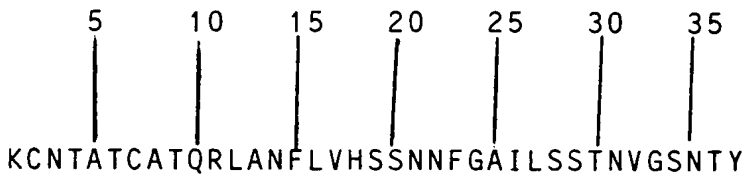
TREATMENT OF DIABETES MELLITUS

Type 1 diabetes mellitus is a disease that affects large numbers of people, and results from the destruction of B-cells within the islets of Langerhans in the pancreas. The current therapy for type 1 diabetes is with parenteral administration of replacement doses of insulin. It is desirable that diabetic control be such that blood glucose levels be returned to near normal in order to avoid the long term complications of diabetes. Such therapy is, however, difficult to control in that it is frequently not easy to avoid the complication of hypoglycaemia, which may lead to morbidity, hypoglycaemic coma, and in infrequent cases to long term brain damage or death.. It has long been known that, for reasons which are not fully understood, hypoglycaemia is a very frequent and very upsetting side-effect of insulin therapy.

Type 2 diabetes mellitus is about 8 to 10 times more prevalent than Type 1 diabetes, and may affect up to 4% of the adult population in Western countries. It is characterized by (1) a deficiency but not an absolute lack of insulin secretion which results in hyperglycaemia, and usually also by (2) varying degrees of resistance to the actions of insulin. In this form of diabetes, unlike Type 1, B-cells are retained in the islets in normal or only slightly reduced numbers. Islet amyloid is also found in most cases (Clark A., Cooper G.J.S. et al., Lancet August 2, 1987).

British Patent Application 8709871 filed 27 April 1987, describes a novel peptide which is identical to or substantially homologous with the amino acid

sequence:



or an active subfragment thereof.

This may alternatively be written using the classical three letter designations of amino acid residues as follows:

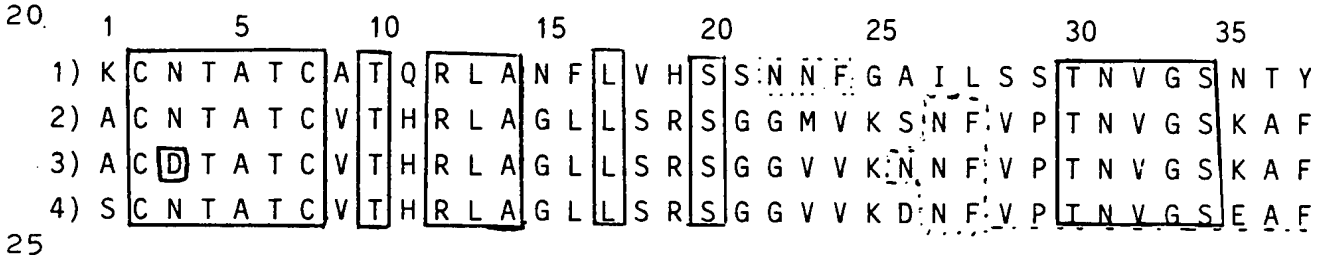
1	5	10	15
Lys Cys Asn Thr	Ala Thr Cys Ala	Thr Gln Arg Leu	Ala Asn Phe
16	20	25	30
Leu Val His Ser	Ser Asn Asn Phe	Gly Ala Ile Leu	Ser Ser Thr
31	35		
Asn Val Gly Ser	Asn Thr Tyr		

This novel peptide, provisionally named diabetes associated peptide or DAP, has been isolated and characterized from the amyloid containing pancreases of type 2 diabetic humans. It is stated that DAP may be found to have clinical utility, such as appetite suppressant activity, and perhaps also vasodilator activity which could be either general activity or be specific for pancreas or islet blood flow.

A peptide that is probably identical to DAP has been described from a human insulinoma, and a highly conserved peptide has been found in the pancreas of a spontaneously diabetic cat (P.Westermark et al., Proc. Natl. Acad. Sci. USA, vol 84, p3881 to 3885, June 1987,

Medical Sciences). This peptide has been named insulinoma or islet amyloid polypeptide (IAPP), but there is said to be little doubt that the human and cat islet amyloid is of identical chemical nature. Immunohistochemical techniques using a peroxidase technique suggest that IAPP is released locally from islet B-cells. Although the roll of IAPP in the islet is stated to be unknown, its conservation and partial identity with CGRP strongly indicate an important regulatory function.

DAP contains thirty-seven amino acid residues and is structurally similar to calcitonin gene related peptide CGRP, having 46% identity with human CGRP-2. The following table compares the primary structure of 1) DAP with that of 2) human CGRP-2, 3) human CGRP-1 and 4) rat CGRP-1. Amino acid identity between peptides is indicated by boxes. Dotted boxes indicate areas of displaced homology



It is known that CGRP exerts significant effects on blood pressure and blood catecholamine levels when administered to rats.

In an article in the Lancet published on 2 August 1987, A.Clark, G.J.S. Cooper et al. report that islet amyloid in twenty-two amyloid-containing type 2 diabetic subjects showed immunoreactivity with antisera to CGRP. This was inhibited by preabsorption of the antisera with DAP, which suggests that DAP is a major

protein constituent of islet amyloid. In addition CGRP/DAP immunoreactivity was found in islet cells of both diabetic and non-diabetic subjects, and preliminary studies show its presence in B-cells. This identification, together with the finding of a similar peptide in insulinomas, suggests that CGRP/DAP may be co-secreted with insulin.

This invention arises from the idea that DAP or DAP-NH_2 or CGRP or a functional peptide fragment of DAP or DAP-NH_2 or CGRP, or a conservative variant of the DAP or DAP-NH_2 or CGRP or fragment, will be of use in the treatment of diabetes mellitus or hypoglycaemia. This idea was unexpected. Although it was known that both DAP and CGRP are associated in some way with diabetes mellitus, it had not previously been suggested that either might be useful in the treatment of the condition. The work on which this invention is based has been concerned with DAP. The invention extends to CGRP on the basis that this peptide has generally similar constitution and properties to DAP, and may therefore be expected to show the same surprising therapeutic effect.

DAP may be in its carboxy terminated form (DAP) or alternatively in its carboxy terminally amidated form (DAP-NH_2). The structure of the amidated form may be represented as:-

$$R\text{-}NH\text{-}(C_6H_4OH)\text{-}CONH_2$$

where R is the residue of the DAP peptide up to the peptide bond to the carboxy terminal residue Tyrosine. Currently known forms of CGRP include a C-terminal amide group which is significant for their biological activity.

By a functional peptide fragment of DAP or DAP-NH_2 or CGRP is meant a peptide fragment at least 5 amino acid residues in length, which performs in vivo a

therapeutic function of the complete DAP or DAP-NH_2 or CGRP peptide. By a conservative variant is meant a peptide which is substantially, though not completely, homologous with DAP or DAP-NH_2 or CGRP or the fragment thereof, but which is functionally equivalent thereto. (See M.O.Dayhoff, A Model of Evolutionary Change in Proteins, in "Atlas of Protein Sequence and Structure", volume 5, supplement 3, National Biomedical Research Foundation, 1978, pages 345 to 352).

According to a preferred aspect of the invention, a composition for use in the treatment of diabetes mellitus or hypoglycaemia comprises a) insulin and b) DAP or DAP-NH_2 or CGRP, or a functional peptide fragment of DAP or DAP-NH_2 or CGRP or a conservative variant of the DAP or DAP-NH_2or CGRP or fragment. The term insulin is here used to cover insulin of natural and synthetic origin and also functional peptide fragments of insulin and conservative variants of insulin or fragments thereof, such as may be used in the conventional treatment of diabetes mellitus.

Products according to the invention may conveniently be provided in the form of solutions suitable for parenteral administration. In many cases, it will be convenient to provide insulin and DAP or DAP-NH_2 or CGRP (or fragment or variant) in a single solution for administration together. In other cases, it may more advantageous to administer insulin, and DAP or DAP-NH_2 or CGRP (or fragment or variant), separately. A suitable administration regime may best be determined by a doctor for each patient individually. It will generally be preferable to formulate such that the molar ratio of insulin to DAP or DAP-NH_2 or CGRP (or fragment or variant) used for the treatment is from 100:1 to 0.1:1. A preliminary study has indicated that, like insulin

immunoreactivity, DAP immunoreactivity and hence DAP, is absent from the islets of Langerhans in type 1 diabetics. It is therefore proposed that the type 1 diabetic syndrome results from a deficiency of not one (i.e. insulin as previously thought) but two (insulin and DAP or DAP-NH_2 or CGRP) hormones. As previously noted, the major problem with insulin treatment of diabetes is hypoglycaemia. It is likely that co-administration of insulin and DAP or DAP-NH_2 or CGRP may avoid this side effect. This may then allow:-

- Tighter diabetic control with reduced risk of hypoglycaemia. This applies to the treatment of type 1 diabetes mellitus, and also for type 2 diabetes mellitus (in the phase of secondary islet cell failure).

- The use of DAP or DAP-NH_2 or CGRP for the therapy of recurrent hypoglycaemia complicating the insulin therapy of type 1 diabetes mellitus (or of type 2 diabetes mellitus).

- The therapy of brittle diabetes (type 1 diabetes mellitus with increased risk of hypoglycaemia).

- The therapy of the intractable hypoglycaemia which may complicate the course of the disease produced by insulin secreting tumours, such as insulinomas.

Although this invention is concerned with results and not with theories, the following explanation of the possible of mode of action of DAP (or CGRP) may be of interest.

1. DAP or DAP-NH_2 is produced in the islets of Langerhans, almost certainly in the B-cell i.e. the same cell that produces insulin. Type 1 diabetes results from the destruction of B-cells in the islets of Langerhans. As these cells contain DAP or DAP-

NH_2, then it is very likely that type 1 diabetes is associated with a deficiency of DAP or DAP-NH_2 as well as insulin. Certainly, DAP or DAP-NH_2 is not seen in the islets of Langerhans in this condition.

2. DAP and CGRP have been shown to modulate the rate of glucose induced insulin secretion from islet B cells in a number of model systems. (Ahren B, Martensson H, Nobin A. Effects of calcitonin gene-related peptide (CGRP) on islet hormone secretion in the pig. Diabetologia 1987; 30: 354-359.)

3. In isolated rat soleus muscles, DAP reduces the rate of glycogen synthesis in both the basal and the insulin-stimulated modes (see Example below).

When 2 and 3 are taken together, DAP (or CGRP) exerts a powerful modulating effect on insulin-induced storage of glucose as glycogen. As this may well be the mechanism whereby insulin resistance is caused in type 2 diabetes, then it may well be that hypersecretion of DAP or DAP-NH_2 (or CGRP) is a factor in the genesis of the insulin resistance found in that condition.

The actions of DAP (or CGRP), as seen above, modulate and reduce the hypoglycaemic effects of insulin, both by reducing the release of insulin in relation to a given glucose stimulus, and (more importantly in the case of type 1 diabetes) by reducing the rate of storage of glucose as glycogen. Hence, DAP (or CGRP) may induce "insulin resistance", and cushion the hypoglycaemic effects of insulin.

EXAMPLE

This experiment was performed to demonstrate 3 above, that DAP reduces the rate of glycogen synthesis in both basal and insulin-stimulated modes.

After having been starved overnight, rats were killed and their soleus muscles extracted and incubated

in buffer at physiological pH. ^{14}C-labelled glucose and cold (unlabelled) glucose were added and the rate of incorportion of glucose into glycogen was measured by extraction of glycogen and counting at intervals of up to six hours. The experiments were done in the presence, 1, 10, 100 and 1000 microunits of insulin per ml. Half the experiments were performed in the presence of 120 nanomoles per litre of DAP.

The results are set out in the accompanying Figure 1, which is a graph of rate of glycogen synthesis against insulin concentration. The open circles represent the results of experiments performed in the absence of DAP; the filled circles represent results of experiments performed in the presence of 15 micromoles per litre of DAP. Each spot at 1 and 100 microunits per ml insulin is the mean of 11 replicate experiments; each spot at 10 and 1000 microunits per ml insulin is the mean of 5 replicates.

At all physiological concentrations of insulin (from 1 to 100 microunits per ml), glycogen synthesis is slowed down in the presence of DAP. The differences are statistically significant (p is less than 0.05 at 1 and 100 microunits per ml by the Mann Whitney U test.

It will observed that the inhibition of glycogen synthesis by DAP persists at low, and presumably even at zero insulin concentrations. It appears that DAP has its own action which is contrary to that of insulin but probably not mediated by direct antagonism of insulin action. In support of this, it has been observed that DAP is not capable of significantly displacing insulin from its receptor on red blood cells.

CLAIMS

1. DAP or DAP-NH_2 or CGRP or a functional peptide fragment of DAP or DAP-NH_2 or CGRP, or a conservative variant of the DAP or DAP-NH_2 or CGRP or fragment, for use in the treatment of diabetes mellitus or hypoglycaemia.

2. A composition comprising a) insulin and b)DAP or DAP-NH_2 or CGRP, or a functional peptide fragment of DAP or DAP-NH_2 or CGRP or a conservative variant of the DAP or DAP-NH_2 or CGRP or fragment, for use in the treatment of diabetes mellitus or hypoglycaemia.

3. A composition as claimed in claim 2, wherein the molar ratio of insulin to DAP or DAP-NH_2 or CGRP (or fragment or variant) is from 100:1 to 0.1:1.

4. A product according to any one of claims 1 to 3, in the form of a solution suitable for parenteral administration.

5. A method of preparing a product for the treatment of diabetes mellitus or hypoglycaemia, which method comprises bringing an active ingredient selected from DAP, DAP-NH_2, CGRP, functional peptide fragments thereof and conservative variants of the DAP or DAP-NH_2 or CGRP or fragment, into the form of a solution suitable for parenteral administration.

6. A method of preparing a composition for the treatment of diabetes mellitus or hypoglycaemia, which method comprises bringing the active ingredients a) insulin and b) DAP or DAP-NH_2 or CGRP or a functional peptide fragment thereof or a conservative variant of the DAP or DAP-NH_2 or CGRP or fragment, into the form of a solution suitable for parenteral administration.

7. A method of treating a mammalian patient for diabetes mellitus or hypoglycaemia, which method comprises administering to the patient DAP or DAP-NH_2 or CGRP or a functional peptide fragment of DAP or DAP-NH_2 or CGRP or a conservative variant of the DAP or

DAP-NH_2 or CGRP or fragment.

8. A method as claimed in claim 7, wherein insulin is also administered to the patient.

9. A method as claimed in claim 8, wherein the insulin and the DAP or DAP-NH_2 or CGRP (or fragment or variant) are administered to the patient in a molar ratio of from 100:1 to 0.1:1.

10. A method as claimed in claim 8 or claim 9 wherein a composition comprising the insulin and the DAP or DAP-NH_2 or CGRP (or fragment or variant) is administered parenterally.

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